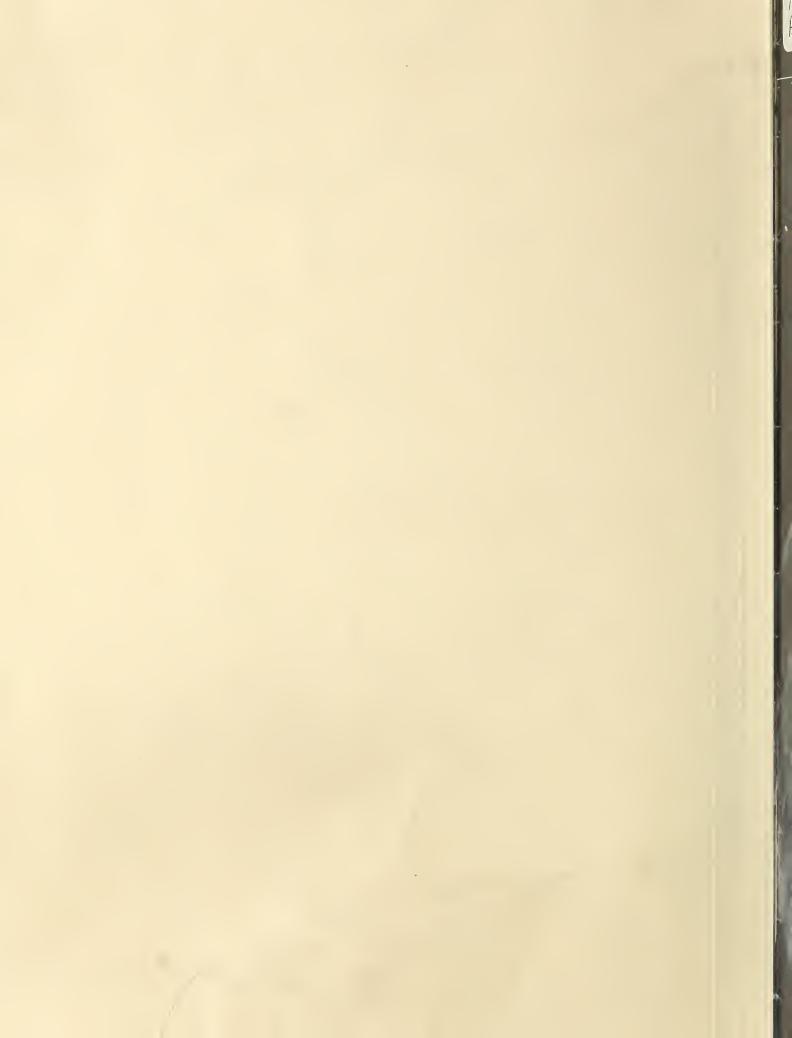
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Agricultural Research Services Agricultural Research Services



Biotechnology and the ARS Role

Three decades ago, agricultural science achieved an extraordinary breakthrough that dramatically increased crop productivity. Millions of people alive today literally owe their very existence to the painstaking progress of traditional plant breeding that brought about the now celebrated "Green Revolution."

Magnificent as that accomplishment was, another revolution is taking form which promises to surpass the far-reaching achievements of classical plant genetics. This revolution is being created by the recent confluence of major discoveries in molecular biology, biochemistry, genetics, and physiology that make progress imminent across a wide front. It will affect every aspect of our food and fiber production.

Biotechnology, as the spectrum of new techniques is known, allows us to understand and alter the processes of living cells.

Many longstanding problems in agriculture may now be solvable through the techniques of gene splicing and regulation, monoclonal antibody research, cell fusion, cell communication within the organism, and membrane research.

We envision more effective ways for increasing a plant or animal's own genetic resistance to pests and other stresses. At the same time, biotechnology should offer improved techniques for discouraging the reproduction or growth of insects, weeds, and other pests. (See Altering Insect Brain Chemistry, p. 8.)

Other new and powerful techniques now make it possible to determine much more quickly the precise chemical makeup of genes and their protein products, telescoping into days what might have taken years to accomplish. Genes can also be located in the chromosomal packages much more easily than in the past.

Scientists are learning to identify genetic material that controls desired characteristics and to move this material between species. In plant science alone, this knowledge will lead to seeds that accumulate more and higher quality proteins and plants that are more efficient in using nitrogen, water, and other scarce resources, that resist drought and diseases, or that flourish in salty water. It will open the door, in time, to plants producing animal proteins or pharmaceuticals. Someday our drugs may be harvested rather than manufactured, thereby giving U.S. farmers a new market for their unexcelled productivity.

Before we savor the fruits of genetic engineering, however, we must identify the mechanisms regulating gene expression-what turns any given gene's activity on or off. To this end, ARS, together with the University of California at Berkeley and the California Agricultural Experiment Station, is establishing the Plant Gene Expression Center. PGEC will assemble experts in the various facets of this field to unravel the complex biology of plant gene expression, stimulate and coordinate complementary research among public and private research groups, and ensure that the knowledge is quickly transferred to the farm.

Headquartered at ARS' Western Regional Research Center at Albany, Calif... PGEC will comprise a core staff of 10 senior scientists, each having support scientists and technicians, to tackle the fundamental questions of gene expression. In addition, through incentive funding, PGEC will establish close cooperation with laboratories that have the necessary scientific expertise and weather patterns to adapt new technologies to specific commodities. The mission of the PGEC consortium will be to convert fundamental research into new tools for solving old problems for agriculture. A board of science advisers-each an expert in an essential phase of the research-will advise and assist the director of PGEC and me in these endeavors.

PGEC will be unique in agricultural science, and we anticipate that it will become a world leader in biotechnology for crop plants. However, the recent federal injunction to halt field testing in California of a genetically engineered bacterium underscores our responsibility

to keep the public informed of the benefits and impacts of this research.

While recognizing that ARS must take full advantage of the new biotechnologies, it is necessary to reaffirm the need for balanced research efforts so that the distinctive problems of plants and animals in agricultural systems can be addressed in both basic research and applied investigations. The glamour of biotechnology must not detract from traditional research that can clearly improve efficiencies in a steady, incremental way.

Applied and basic research in agriculture are so intimately intertwined in the biotechnologies that many practical spinoffs may be put on line while fundamental inquiries continue to pursue the basic knowledge on which the next round of major advances in agriculture will be built.

Meeting the technology challenge of the next decade will require a major commitment of effort from ARS and its partners in research—the land-grant university system, including the state agricultural experiment stations, and the private sector, including industry and the research foundations.

> Terry B. Kinney, Jr. Administrator

Editor's note: In this and the next two issues, Agricultural Research will feature current basic research upon which the new technologies will be built. The September issue will survey biological membranes and their role in the success of genetic engineering. In the October issue, we take a closer look at biological messengers—those chemicals that transmit signals within and between organisms.

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John R. Block, Secretary U.S. Department of Agriculture

Orville G. Bentley Assistant Secretary Science and Education

Terry B. Kinney, Jr. Administrator Agricultural Research Service

Cover: The female corn earworm moth (Heliothis zea) looks innocent enough, but she can produce 500 to 3,000 off-spring that, together with their cousins, consume nearly 4 million acres of corn and other field crops each year and another 40,000 acres of row crops—in spite of the use of pesticides. The secret of her reproductive success lies behind the bulging eyes, in her brain. ARS scientists in five laboratories around the country are learning how insect brain chemistry ensures survival of such pests. Story begins on page 8. (0884X1083-14)

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Critical Knowledge of Mold Life Cycles Gained

Microbiologist Donald Wicklow examines toxin-producing molds in his laboratory at the Northern Regional Research Center, Peoria, III. (0180X065-34A)

The discovery in corn and soil of sclerotia—a dormant survival stage—of two toxin-producing mold species fills blanks in the molds' life cycles.

"Recognizing sclerotia as natural states in the life cycles of these species is like knowing that the tuber is a means of propagating the potato," says Donald T. Wicklow, microbial ecologist at the Northern Regional Research Center, Peoria. "The importance of Aspergillus flavus as a colonist of preharvest cereals and other farm products makes it imperative to know the role of the sclerotium in the life cycle of A. flavus."

The research demonstrates that sclerotia on the soil surface may be the primary source of molds growing in corn kernels. "The prospect of eventually controlling A. flavus increases dramatically if we find that soilborne sclerotia represent the primary inoculum," Wicklow says. Various agronomic practices have been used to reduce losses caused by other sclerotium-forming molds that infect plants.

Although mycologists first reported *A. flavus* sclerotia in laboratory cultures almost 80 years ago, no one reported finding the dormant structures in nature or questioned their function.

Leading a multidisciplinary team, Wicklow found the first naturally occurring sclerotia of *A. flavus* and *Eupenicillium ochrosalomeum* in kernels from standing and downed corn and in combine debris and soil from Georgia fields. The team also demonstrated that sclerotia can overwinter in soils near Dawson, Ga., but not Peoria, Ill. Other team members are Richard J. Cole, Bruce W. Horn and Janine E. Donahue, ARS, and William R. Burg, University of Cincinnati.

A. flavus can produce aflatoxin, a suspected carcinogen in certain animals. The pencillium mold can produce citreoviridin, a toxin affecting the nervous system. The team found the first citreoviridin reported in cereals in the field when they found the sclerotia in standing corn.

"The sclerotium serves as an important survival structure in the life cycle of several important plant-disease fungi," Wicklow says. Molds, which are fungi, are masses of microscopic threads, or mycelia, that penetrate the nutrient material—a corn kernel, for example. The mycelia stop growing and harden to form sclerotia, which can endure unfavorable conditions. Sclerotia remain dormant until conditions become favorable, then grow again.

"Spreading sclerotia along with husks and other plant debris on the soil surface could lead to a buildup of inoculum in fields that are continuously cropped to corn or rotated between corn and other susceptible crops such as cotton and peanuts," Wicklow says. "We found more sclerotia in chaff and debris in the fields where mold infestation was greatest before combining."

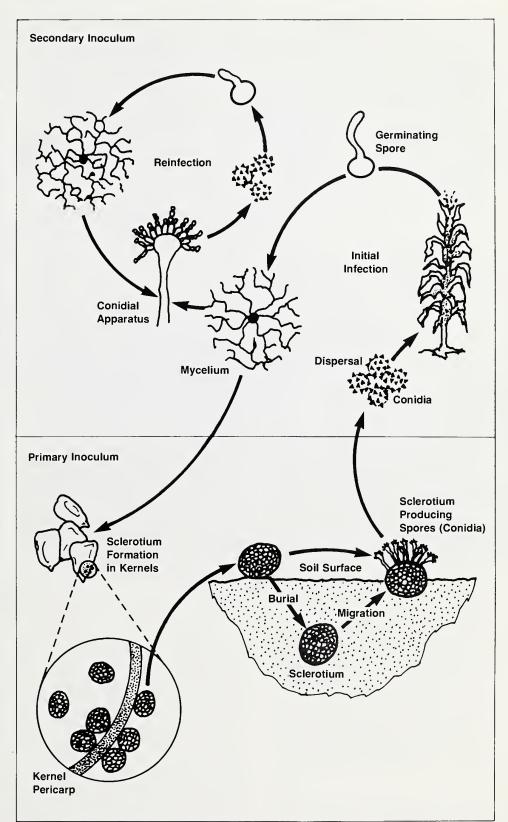
Finding sclerotia in corn and in combine debris underscores the need to determine the possible connections between sclerotia on soil and in the corn ear. Wicklow and Horn studied one condition, husk tightness, that might affect sclerotium production in damaged kernels. To control the environment and eliminate variables, they grew corn in a growth chamber at the University of Wisconsin and inserted toothpicks through husks to inoculate kernels with A. flavus.

The mold "grew and formed large numbers of sclerotia in wound-inoculated preharvest corn," Wicklow says. "More sclerotia formed in wounded kernels of a loose-husked northern hybrid than in a tight-husked southern hybrid." Most sclerotia formed on the wounded kernels. "In a field situation," Wicklow points out, "one heavily insect-damaged ear might be a source of thousands of mold sclerotia that would be spread on the soil at harvest."

Sclerotia can germinate in soil if they are not completely buried. Wicklow and Donahue distributed *A. flavus* sclerotia on the surfaces of sand and of garden soil and buried some in soil. Mold spores were produced on exposed surfaces of the sclerotia. "Sclerotia buried for 10 days germinated within 72 hours after flooding washed them to the soil surface," Wicklow says.

Donald T. Wicklow is located at the Northern Regional Research Center, 1815 N. University, Peoria, III. 61604.

—Dean Mayberry, Peoria, III. ■



A sclerotium of the aflatoxin-producing mold, Aspergillus flavus, lies partly uncovered at the soil surface (lower right) producing conidia (spores). Some conidia reach corn kernels and grow, forming mycelia. Mycelia produce more conidia, which form more mycelia (upper left), or harden to form sclerotia (lower left), which reach the soil surface. Sclerotia that are not completely buried can germinate and produce spores, completing the mold's life cycle. (PN-7125)

Textiles That Turn On to Temperature

Textiles with a built-in thermostat may someday warm you when you're cold and cool you when you're hot—in clothing and housing insulation. Such materials could also protect animals, plants, and food from wide temperature variations. That is the result of an ARS search at the Textiles and Clothing Laboratory, Knoxville, Tenn., for treatments that enable textiles to adapt to temperature.

Laboratory director Tyrone L. Vigo and chemist Cynthia M. Frost have found two organic alcohols that consistently make textiles absorb or release up to four times as much heat as ordinary textiles at temperatures ranging from 41° to 212°F. The modified textiles retained these characteristics through more than 50 heating and cooling cycles, says Vigo. He and Frost have applied for a patent on the treatment.

The three fiber types tested—rayon, cotton, and polypropylene—range from very high to very low water content. Polypropylene is commonly used in carpets, in nonwoven interlinings, and in geotextiles for highway reinforcement and swimming pool enclosures.

The two organic alcohols—DMP and HMP for short—belong to a unique group of chemicals called plastic crystals that can undergo large changes in heat content while still in the solid phase; that is, without melting and resolidifying. DMP increased the heat content of rayon fibers 3.5 to 4.0 times more than untreated fibers, but HMP did not produce consistent results with rayon. Both compounds enhanced the heat content of cotton and polypropylene 2.5 and 2.0 times, respectively.

The alcohols are commercially available, says Vigo. DMP is used in the manufacture of polyester and urethane foams and as a lubricant. He expects that these compounds will be applied to fabric as a finish much like durable press finishes are applied now. However, several practical problems remain to be solved before temperature-adaptable fabrics can be used in commercial clothing.

The most basic one is protecting the plastic crystals from water, because they are water soluble. In fact, they were applied to the fibers in an aqueous solution, then dried. Vigo and Frost are working on a waterproofing finish for treated fabric or a process to encapsulate the plastic crystals into the fiber or fabric.

Weight could be another problem for clothing, because the treatment doubles the weight of the fabric. It is far less bulky than insulation or goose down, however.

Also, the lifetime of the material must be determined, says Vigo. The Knoxville chemists plan to evaluate the plastic crystal-treated fibers for durability and performance.

Regardless of the problems, plastic crystals appear to be superior to the 1971 invention by a textile manufacturer that fills hollow fibers with a mixture of liquid and gas, says Vigo. As the temperature changes, more or less gas is released, altering the insulation value of the fiber. It is based on a volume change, with the gas providing dead air spaces similar to insulation and down, he explains, whereas the new process is based on a change in heat content of the crystals themselves.

Besides clothing, plastic crystals could be used to manufacture materials to insulate buildings and to protect plants, animals, and food from wide swings in temperature. They act as a "buffer against temperature extremes," says Vigo. Textile manufacturers, chemical companies, and those who make plastic crystals have already shown interest in the process, he says.

Still, this is just one of a number of novel materials that clothing designers of the future will have to work with, the textile expert says. "Just as we have seen a proliferation of specialty chemicals designed for very specific purposes, I think we will see the introduction of a growing number of specialty fibers."

Tyrone L. Vigo and Cynthia M. Frost are located at the Textiles and Clothing Research Laboratory, 1303 W. Cumberland Ave., Knoxville, Tenn. 37916. —Henry Becker, Beltsville, Md. ■





Fibers in untreated cotton fabric (above, PN-7117) appear regular compared with those in the same fabric treated with polyethylene glycol-600 (below, PN-7118). This common chemical is comparable in its thermal properties to plastic crystals and a less expensive alternative. Unlike plastic crystals, however, polyethylene glycol undergoes a phase change (liquid-solid) when storing or releasing heat. Micrographs courtesy of University of Tennessee. Knoxville.

Glenn Burton: More Millet for the Sub-Sahara

Glenn Burton doesn't have time to think about retiring from his 48-year career with the Agricultural Research Service. "There's too much that needs to be done," he explains.

The plant geneticist maintains a regime that belies his 74 years. If he's not consulting somewhere in the world, or conducting a college seminar, he's likely to be found in a greenhouse or testing a new variety of pearl millet in an experimental plot.

In keeping with Burton's interests in both agriculture and humanitarian service, it is not surprising that he is again involved in a project that may lessen hunger for millions of people living in the semiarid regions of the developing world.

Last May, under the sponsorship of the International Board of Plant Genetic Resources, Burton went to Bourkina Fasso (formerly Upper Volta) to initiate a 3-year project to collect and evaluate pearl millet germplasm.

Half that seed will be evaluated in Bourkina Fasso. In the past, it was customary to grow and evaluate seed outside its native country. The remainder will undergo simultaneous testing at ARS's Plant Germplasm Quarantine Center at Beltsville, Md., the Coastal Plain Experiment Station at Tifton, and at the International Crops Research Institute for the Semiarid Tropics in India.

Bourkina Fasso, located in West Africa at the southern end of the Sahara Desert, depends heavily on pearl millet. The grain is the country's major cereal crop and a staple in the national diet. With annual population growth significantly outstripping the increase in food production, it is urgent that improved land races be identified and more widely cultivated. Another problem with which the nation must contend is the shortage of water. As part of the semiarid tropics, Bourkina Fasso has an annual rainfall of only 20 to 30 inches—most of which falls in a single rainy season.

Speaking of the project's prospects, Burton says: "I think there is a good chance that one of the land races may be enough better to immediately become an improved variety. There is no guarantee, of course, but I think there's a good chance."

If so, it may not be serendipity—a favorite word of Burton's. He has a knack



At the University of Ouagadougou, Bourkina Fasso (formerly Upper Volta), ARS plant geneticist Glenn Burton (left) inspects a race of pearl millet with Michael Horne of the U.N. Food and Agriculture Organization. Idrissa Ousmane (right), a teacher at the University's agricultural college, initiated the millet-improvement project while a graduate student at the University of Georgia. (PN-7122)

for knowing about pearl millet, having created new hybrids of the plant that help feed millions of people around the world. In 1961, Burton sent his cytoplasmic male-sterile millet seeds to India for use in their breeding programs. By 1965, Indian scientists had developed from Burton's seeds a new hybrid that yielded 88 percent more grain than common millet.

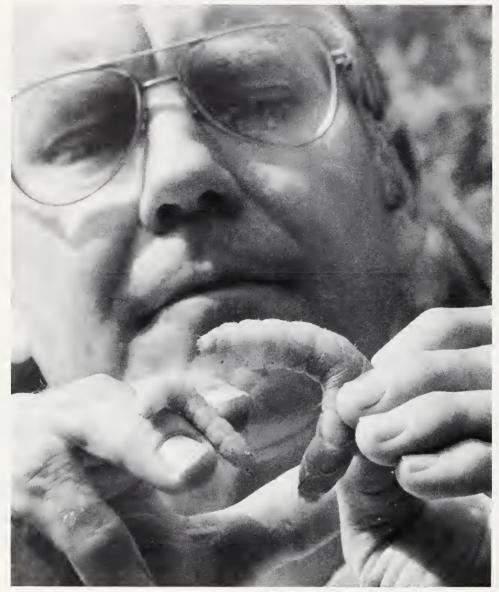
Burton has received both the National Medal of Science and the President's Medal of Honor for his work in developing nutritious grasses for people and beef and dairy animals in the Third World.

Although Burton has spent his entire career with ARS at the Coastal Plain

Experiment Station, he actually has two employers. He is also a salaried faculty member of the University of Georgia. Shortly after Burton came to the Tifton station, which is staffed by ARS personnel as well as University of Georgia scientists, the university requested USDA's permission to help pay Burton's salary because of his contributions to the university's agricultural research program.

Glenn Burton is located at the Georgia Coastal Plain Experiment Station, Tifton, Ga. 31793. —Jack Reeves, news editor, University of Georgia, Department of Agricultural Communications, Athens, Ga.

Altering Insect Brain Chemistry— An Emerging Science



James Svoboda, chief of the Insect Physiology Laboratory, Beltsville, Md., exhibits a stunted tobacco hornworm (left) beside its normal cousin. The stunted hornworm was fed one of the several compounds developed by Svoboda and coworkers that prevent insect larvae from producing molting hormone. (0684X852-16)

An insect may drop dead if a certain chemical is altered in its brain. Another insect may outdo itself—bees may pollinate crops and flowers longer than usual. Whether the insect dies or excels, the altering of behavior is wrapped up in insect neurochemistry.

The connection has intrigued researchers for years, but "advances in technology and molecular biology are helping us look at insect neurochemistry in a whole new way," says ARS pest management specialist J.E. (Bud) Wright, who has studied insect behavior for 18 years.

"An insect's brain and nerve trunks form a vast chemical pathway for the signals that dictate behavior. It's the chemistry in the brain we want to exploit," says Wright. If researchers can disrupt the chemistry, then the insect's regular chemical messages jam up or conflict. It's akin, in a way, to breaking an enemy's code and relaying false information. The false signals can cause havoc. They can halt the molting of the skeletal shell, upset mating, realign hormones, or prompt other fatal reactions.

Insect neurochemistry will one day produce a new class of safer insecticides



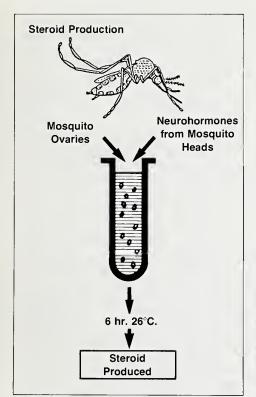


Ovary (above) from a female mosquito that had been decapitated shows no yolk indicating a lack of egg development. (PN-7120) When she is injected with neurohormones extracted from mosquito heads, egg development ensues as indicated by the presence of white yolk. (PN-7121) Micrographs by insect physiologist Thomas Kelly, Beltsville, Md.

that will alter the behavior of insect pests without harming other living things, predicts ARS administrator Terry B. Kinney, Jr.

Agricultural and environmental problems have evolved from simpler perceptions of pest management in the recent past, says Kinney. The surprising ease with which many insect pests have developed resistance to chemicals; cases of pesticide residues found in groundwater, waterways, and food; and some modern farm and marketing techniques that give pests new advantages are all part of today's problems. Insect neurochemistry has already shed some light on why insect pests historically have sidestepped our best shots at managing them.

The science of neurochemistry can be traced back 73 years when Stefan Kopec, a Polish entomologist, demonstrated that a factor in the brain of the gypsy moth larva controls its development. This was one of the earliest evidences of chemical communication between the nervous and endocrine systems in animals. A little more than 20 years later, British en-

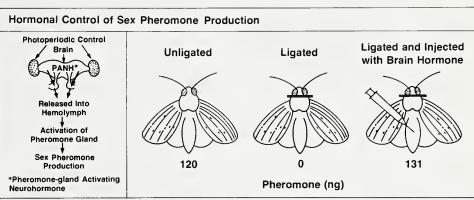


Neurohormones are crucial to egg development as depicted in this bioassay done by insect physiologist Edward Masler at the Insect Reproduction Laboratory, Beltsville, Md. When combined with immature ovaries, neurohormones stimulate the ovaries to produce a steroid essential to egg development. (PN-7123).

tomologist Sir Vincent Wigglesworth put forth the notion that insects produce a hormone to help them molt—the periodic shedding of their outer shells that allows for growth. Scientists have since discovered numerous types of molting hormones as well as juvenile hormones that regulate metamorphosis. Recently, they have identified the brain hormones that orchestrate the production of other hormones.

Almost all scientific data in brain chemistry have been gathered in the past 5 years. Rapid advances in laboratory tools and techniques, including microsurgery on an insect's brain, have given scientists the means to find, trace, and study hormonal compounds in amounts as little as 1 picogram—one-trillionth of a gram. By contrast, medical and veterinary neurochemists work with hormones isolated in full gram amounts from glands or cells in the brain.

Molecular biology and a trend to multidisciplinary research have also opened



The normal process of sex pheromone production in female moths is shown in the box. A ligated female moth injected with brain hormone produces approximately the same amount of pheromone as an unligated moth. The ligated moth, however, produces no sex pheromone. (PN-7124)



At the Organic Chemical Synthesis Laboratory, Beltsville, Md., this female corn earworm moth (Heliothis zea) is being injected with hormones extracted from another female's brain. This will restore her ability to produce sex pheromone. Pheromone production had been stopped by a technique called ligation in which a string tied between the head and thorax prevented the release of brain hormone into the hemolymph—the insect circulatory system. (0684X768-7A)

new opportunities to understand the intricacies of the insect brain and its effect on behavior, says Wright.

Just how fast scientists can translate neurochemistry into pest control technology is still to be answered, says Wright, noting that only a handful of researchers at ARS laboratories and universities are pursuing indepth studies. However, in-the-field technology to interfere with insect mating signals, interrupt egg or sperm production, or disrupt key steps in life cycles is now feasible.

ARS' most concentrated research in insect neurochemistry takes place in five laboratories where scientists study how the insect brain controls its reproduction, growth, and vital functions.

J.E. Wright is on assignment to the ARS National Program Staff, Rm. 232, Bldg. 005, Beltsville Agricultural Research Center—West, Beltsville, Md. 20705.

Playing the Numbers Game

The Insect Reproduction Laboratory, Beltsville, Md., is one of the few facilities in the world devoted to the intricate hormonal systems associated with insect mating and breeding. The laboratory supplies scientific data to a wide community of researchers who explore ways to harness the most menacing aspect of an insect pest: its numbers.

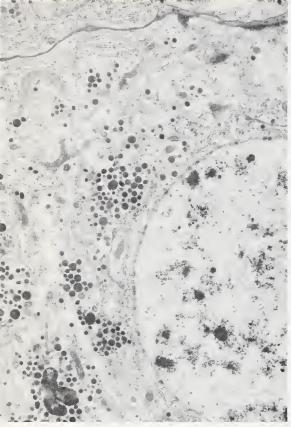
"It seems strange," says laboratory chief Alexej B. Borkovec, "that despite the insect's power to reproduce rapidly, even explosively at times, serious research to interfere with that power began little more than 25 years ago."

While Borkovec and coworkers had identified neurohormones that trigger egg production in females of some species, systems in male insects that produce sperm had remained a mystery. Recently, however, physiologist Marcia Loeb discovered that steroids are produced in the testes of a moth species, indicating that a male regulatory system does exist—one that could be exploited in future pest control strategies.

Borkovec projects that products or control schemes to upset reproductive cycles could be a routine part of insect management in another 25 years.

The common house fly is helping scientists at the Metabolism and Radiation Research Laboratory, Fargo, N. Dak., study roles played by hormones in fly and mosquito reproduction.

Operating with a microscope and tiny surgical tools, entomologist Terrance



Dark, round granules laden with neurohormones (neuropeptides) are visible inside a cell from the brain of a stable fly at 16,000 times life size. The cell specializes in producing and secreting these hormones that initiate and coordinate physiological processes in the fly. Micrograph by entomologist Shirlee Meola at the Veterinary Toxicology and Entomology Laboratory, College Station, Tex. (PN-7119)

Adams removes the ovaries, brain, or other hormone-producing tissues from the body of a house fly. He then uses cell and organ tissue culture methods pioneered by colleague Edwin Marks to study the physiological effects of each hormone in isolation from other insect tissues. Most hormones have the effect of changing rates of cellular processes some distance from where they are manufactured, such as in the brain. Adam's work on isolating hormone actions is essential to sorting out common interactions of neurohormones between points of origin and effect.

Certain neurohormones control the ability of insects to produce sex pheromones—chemical signals that one sex, usually the female, produces to "call" a mate. The first sex pheromone identified was that of the silkworm moth,

25 years ago. Scientists then recognized that sex pheromones could be used in insect traps and to disrupt mating patterns of insect pests.

As a result, the sex pheromones for more than 300 insect species are now known. Over 100 are used in insect traps for monitoring populations of pests, and at least 12 are registered with the U.S. Environmental Protection Agency as pest control chemicals.

How do insects make pheromones? The answer is emerging through research in neurochemistry. One clue was discovered recently at the Organic Chemical Synthesis Laboratory, also at Beltsville, where the pheromones of many insect pests have been identified.

Entomologists Ashok Raina and Jerome Klun found a hormone, produced in the brain of the corn earworm moth, that causes a gland in the tip of the female moth to emit a sex pheromone. But before the scientists can find a way to shut down production of the pheromone by tampering with the new hormone, they must sort out hormonal interactions that are still unknown.

Alexej Borkovec is located at the Insect Reproduction Laboratory, Rm. 109, Bldg. 306, Beltsville Agricultural Research Center—East, Beltsville, Md. 20705; Terrance Adams is at the Metabolism and Radiation Research Laboratory, P.O. Box 5674, Fargo, N. Dak. 58105; and Ashok Raina and Jerome Klun are at the Organic Synthesis Laboratory, Rm. 333, Bldg. 007, Beltsville Agricultural Research Center—West, Beltsville, Md. 20705.

Stunting Growth

Molting is essential to the growth and metamorphosis of all insects. It is therefore a logical point of attack. At the Insect Physiology Laboratory at Beltsville, Md., seven molting hormones have been discovered and their complex chemistry deciphered. Scientists there are finding ways of interfering with hormone production in insects.

A key factor in many plant-feeding insects is that production of molting hormone depends upon insects' metabolizing sterols from the plants they have eaten, explains laboratory chief James Svoboda. With hormonal signals from the brain, the insects convert these plant sterols into cholesterol that, in turn, is

converted to a molting hormone ecdysone. Svoboda and colleagues have synthesized a number of compounds that interfere with steroid metabolism.

Agency scientists are experimenting with some of these metabolic steps so as to disrupt the molting cycle or reproductive processes.

James Svoboda is located at the Insect Physiology Laboratory, Bldg. 467, Beltsville Agricultural Research Center— East, Beltsville, Md. 20705.

Jamming Vital Functions

Many of the vital functions of insects are ultimately controlled by muscles that are stimulated by neurohormones secreted by the nervous system. The Veterinary Toxicology and Entomology Research Laboratory in College Station, Tex., is focusing on the neuropeptides that control digestion, excretion, and egg deposition.

Entomologists Mark Holman, Renee Wagner, and Benjamin Cook have now purified several of these peptides. When their structure has been determined and copied in the lab, says Cook, it will be possible to define how they control vital processes down to the chemical reactions involved. Such an objective also depends on knowing the fine structure of the insect cells that synthesize and secrete the neuropeptides as well as those that respond to them. Entomologist Shirlee Meola is using electron microscopy, both conventional and scanning, to uncover the neurosecretory relationships.

Once these objectives are in place, scientists will be able to prepare analogs—chemicals that are similar enough to the natural neuropeptides to be mistaken for them but different enough to upset the chemical machinery. Previous work on another insect neuropeptide has shown that all insects and some of their relatives use the same chemical, Cook says. The neuropeptide analogs should provide new tools for insect management.

Benjamin J. Cook is located at the Veterinary Toxicology and Entomology Research Laboratory, P.O. Drawer GE, College Station, Tex. 77841.

—Stephen Berberich and Bruce Schwartz, Beltsville, Md. ■

VGS: An Old Hydraulic Principle Works for New Sewers

"It's such a simple idea, you wonder why you didn't think of it before," says engineer Jerry Newman.

He is talking about a novel approach to disposing of residential sewage that he and engineer John Simmons and their predecessors at the ARS Rural Housing Research Unit, Clemson, S.C., worked on for more than a decade.

In full, the system is called a small-diameter, variable-grade, gravity sewer—or VGS. The VGS differs from a common gravity sewer by carrying only waste water from a property to a central sewage-treatment facility. Solids and floating substances, such as grease, are left behind in a septic tank buried on the property to be digested—converted to liquid form—by bacteria. Just the liquid discharged from septic tanks enters the sewer system.

After their years of work on the concept, Newman and Simmons have reason to be gratified: two counties in eastern Ohio have VGS projects underway. For the citizens of these counties, VGS will mean multimillion-dollar savings (see box, next page). For the nation, Newman believes, VGS could save taxpayers billions of dollars over the next few years.

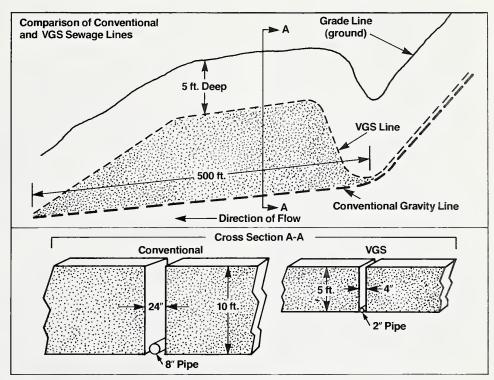
Less excavation, easily installed pipe, no pumps, and virtually no need for maintenance are the reasons behind the figures.

Experimental System for a New Community

"The impetus for our research,"
Newman says, "was Public Law 89-240, passed in 1965. That law authorized USDA's Farmers Home Administration to make loans and grants to rural areas for sewers. But it soon became apparent to FmHA engineers that conventional sewers would be too expensive to build in areas with small or sparse populations. As USDA's research agency, we agreed to try to find a less costly alternative."

The experimental system was constructed in Mount Andrew, Ala., as part of an FmHA-financed subdivision sponsored by the Tuskegee Institute.

"We didn't say, 'Let's build a variablegrade gravity sewer,' "Newman says. "We actually started from a different theoretical base. Some of the theory didn't work out, but the sewer worked



The difference between a variable-grade and a conventional gravity sewer is shown by a section of grade profile, Muskingum County, Ohio, where a sewer line is to be laid. The VGS line will follow the contour of the ground at a 5-foot depth; a conventional sewer line would have to be laid at a straight 10-foot depth to keep the line running downhill. The cross sections contrast the size of ditch that needs to be excavated for VGS and conventional sewer lines. Profile courtesy of Design Enterprise Ltd., Dayton, Ohio. (PN-7127)

anyway. What we had was probably the first true VGS in the country—a lot of the explanation of why it worked came later."

Nevertheless, the primary goal of reducing costs had been achieved: the sewer lines had been laid at a cost of less than \$2 a foot. Even under the best circumstances, Newman says, regular sewer lines would have cost \$20 to \$25 a foot.

How It works

Having a system carry only water opens up possibilities for a simpler, less expensive sewer design. Not having to accommodate solids, the sewer lines can be smaller and made of lightweight, easy-to-install plastic. There is no need for cleanouts and manholes because there is no material to plug the system up. Most important of all in lowering costs, deep excavation to keep the sewer lines headed downhill is not necessary: they can be laid at shallow depth following the contour of the land, uphill and down.

To understand how it works, think of the U-shaped trap under the kitchen sink. Water always stands in the trap (to block sewer gases from entering the house). When water is poured down the drain, it overflows into the drainpipe rather than backing up into the sink, because the discharge point from the trap is lower than the input point. Backups occur only if there's material clogging the U-trap.

The VGS works the same way. As long as each septic tank outlet on the sytem is higher than the entire system's outlet, generally a central sewage-treatment facility, the sewer lines will continue to drain, although there will be standing water in low points during periods of no flow. Because the sewer carries no solids, low points cannot become blocked as they would in a conventional sewer system.

"In the 9 years since we installed it,"
Newman says, "the Mount Andrew
system has functioned without any problems, and we have dug up parts of it to
look."

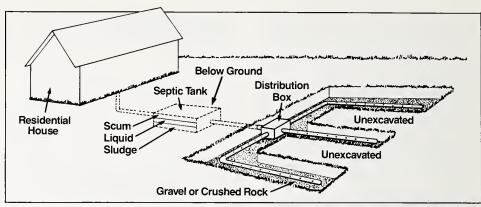
But aren't septic tanks a complication that could wipe out the savings gained by variable-grade sewer lines?

"In the first place," Newman says, "in many established communities where sewer systems will be installed in coming years, septic tanks already exist. We believe that many of them can be used with VGS, perhaps after slight modification. In new communities, the cost of putting in a septic tank as part of a VGS during house construction would be far less than building a conventional gravity sewer without septic tanks.

"Septic tanks do have to be pumped out every 3 to 5 years. Sludge, which is the residue from bacterial digestion of organic material, settles to the bottom and has to be removed periodically. But you've got to remember that sludge is a byproduct of any sewage treatment, so whether it accumulates in individual septic tanks or at a central plant, it's got to be removed and disposed of. So, no, we don't think of septic tanks as a complication. Collection from septic tanks may even be an advantage because they'll usually be found in outlying areasnearer sludge disposal sites than most central treatment plants are."

The System's Potential

An idea of the U.S need, in terms of cost, for sanitary sewage-collection systems can be gotten from the estimates of the U.S. Environmental Protection Agency. According to EPA's Needs Survey for 1982, the latest made,



The conventional septic tank-leach field system shown here is a satisfactory way to treat household sewage if the amount of water usage, soil type, topography, water table, and size of the lot are all in proper relation. In a VGS system, a modified septic tank discharges liquids into sewer lines for final treatment at a central facility. (PN-7126)

13,000 communities are without central sewage collection and treatment facilities and rely on septic tanks and leach fields or less. The cost, in 1982 dollars, of building conventional collector sewers for these communities was estimated at \$21 billion.

These figures do not include substantial new housing developments built since 1972 or communities falling outside the jurisdiction of an organized sewer authority. Rather, they represent only the needs of communities existing in 1972 that are eligible for funding under the Federal Water Pollution Control Act. But EPA's estimates are not just conjecture.

By the year 2000, according to the EPA survey, 8,400 of the 13,000 communities plan to have sewers built.

Estimates of how much VGS could save these and post-1972 communities in construction costs range from the 36 percent for the two Ohio projects discussed (see box), to an optimistic 50 percent. Wherever actual costs for a specific community might ultimately fall within that range, it is clear that VGS has the potential of saving billions, as Jerry Newman thinks.

VGS is an idea public-works officials should look into when they contemplate building a new sewer, extending an ex-

Two Ohio Counties Ready To Try VGS

The risk of departing from timetested, conventional sewage systems is begin taken in Tuscarawas and Muskingum Counties, Ohio, where several publicly owned VGS projects, the first in the nation, are underway.

In one sense, these sewers can be viewed as demonstration projects on which the future of VGS may rest. Unlike the experimental Mount Andrew installation, these are systems that have had to win the approval of local authorities, state environmental and health officials, and lending and granting organizations.

In Tuscarawas County, the engineering firm of W.E. Quicksall and Associates, Inc., New Philadelphia, is designing a VGS for the villages of Midvale (pop. 654) and Barnhill (pop. 327). The sewer will be about 7.4 miles long and serve 323 homes.

"We first planned a conventional gravi-

ty sewer," says project engineer Donald R. Quicksall, "but the estimated 1982 cost of \$3.3 million was not economically feasible for an area where the average median household income was \$12,856 in 1980. So we went back to the drawing board. At 1984 prices, we estimate that a VGS can be constructed for about \$2.1 million, and 85 percent of this cost should qualify for funding from the U.S. Environmental Protection Agency's innovative and alternative technologies grant program."

Each household in Midvale and Barnhill already has a private sewage-disposal system. As in many other communities in the country, these systems are no longer adequate. The lots are too small, water usage is too large, and the soil and topography are not suitable for properly working leach fields. Septic tank effluent is discharged into creeks and streams or

on the ground, contaminating the water and fouling the air. VGS will eliminate these problems at a cost the citizens can bear.

Similar problems will be solved by VGS in nearby Muskingum County, where the engineering firm of Design Enterprise Ltd., Dayton, has three projects in various stages of design. These projects are mixed systems in which industrial and commercial users will be served by conventional gravity lines and residential users by variable-grade lines and septic tanks. According to the firm's president, Frank Leone, Muskingum County, except for the most rural parts, will be completely sewered for the first time when the projects are complete in 1986 or 1987.

Because none of the projects had actually been let out for bid at the time this article was written, Leone was reluctant to speculate about dollar costs, but he

AgResearch Notes

isting one, or replacing an old one.

Jerry O. Newman and John D. Simmons are located at the Rural Housing
Research Unit, P.O. Box 792, Clemson,
S.C. 29633.—David Pyrah, New Orleans
La. ■

For Further Information

John D. Simmons and Jerry O.
Newman have written "Design of Small-Diameter, Variable-Grade, Gravity
Sewers" for engineers and public-works professionals. The 16-page publication is available from: Superintendent of Documents, Government Printing Office, Washington, D.C. 20402. The stock number is 001-000-04412-5, and the price per copy is \$1.25.

USDA's Farmers Home Administration makes loans and grants for sewers to communities with less than 10,000 population. Information may be obtained from any of FmHA's state, district, or local offices.

The U.S. Environmental Protection Agency will fund 85 percent of the cost of eligible VGS projects under its innovative and alternative technologies grant program. Information may be obtained from any of EPA's regional offices or from state environmental agencies.

expected that the savings attributable to VGS would be substantial. For example, he estimated that the VGS cost of one of the projects, known as Maysville III, would be about 36 percent less than the cost of a conventional gravity sewer. The VGS portion of Maysville III will be about 19.5 miles long and serve about 900 households, so the amount of money involved will obviously be considerably larger than the cost of the Midvale-Barnhill project.

"VGS is the answer to some problems in Muskingum County," Leone says. "It's rolling, hilly, strip-mine country and not very densely populated. The people need lower cost options to have a sewer system at all. In addition to qualifying for 85-percent funding from the EPA, two of the projects have qualified for some funding from FmHA."—D.P.

A Bird's-Eye View of the Embryonic Turkey

Since 1974, scientists have been able to observe chicken embryos develop outside their shells—something not possible with other animals. This capability may expedite research into embryonic life in larger animals and humans, says ARS animal nutritionist Mark P. Richards.

At Beltsville, Md., Richards now keeps turkey embryos alive in culture dishes up to 27 of the 28 days required for incubation. He is studying how they metabolize several trace minerals.

Use of culture dishes allows the researcher to follow an embryo's development and to study the physical and chemical changes that accompany development. It also permits easier access when it's time to take tissue samples, make injections, graft tissues, or perform other operations, says Richards. He predicts that "shell-less poultry embryos could become an alternative in research to gain knowledge of how embryonic nutrition relates to human ailments, possibly even birth defects."

To prepare the embryos, Richards first incubates fertile eggs for 4 days. After cleaning the shells with alcohol, he carefully scissors them in half and empties the eggs' contents into culture dishes, making certain each embryo and its membranes face upward. A standard laboratory incubator maintains the environment as it would be inside the eggshell.

Blood levels of the trace minerals zinc, copper, and iron were similar in embryos whether they had shells or not. However, there were important developmental differences between the two groups. Body weights held fairly close for 17 days, then those of the shell-less embryos lagged behind. Richards attributes this to a combination of lower calcium and protein levels in the blood and abnormal trace mineral metabolism. Cultured embryos cannot draw upon the calcium in shells that otherwise would be availble for normal growth, he explains. And they apparently do not draw upon albumin protein as their normal counterparts do.

After 19 days, calcium-deficiency abnormalities began appearing in some of the shell-less embryos. Beaks did not



Sixteen-day-old shell-less turkey embryos are being injected with a metal solution by animal nutritionist Mark Richards, who is studying how the embryos metabolize trace elements. (0684X765-19A)

elongate properly, skulls were soft, and feathers did not open or expand fully. Altered trace mineral metabolism may also contribute to the abnormal feathers, says Richards.

By refining the use of calcium supplements and antimicrobial drugs, he says, it may be possible to grow the shell-less embryos right through hatching.

Mark P. Richards is located at the Non-Ruminant Animal Nutrition Laboratory, Bldg. 200, Beltsville Agricultural Research Center—East, Beltsville, Md. 20705.

-Russell Kaniuka, Beltsville, Md.

Christmas Trees Thrive in Desert Country

Growing Christmas trees in the Arizona desert with its limited precipitation and 100-degree temperatures may seem like an effort in futility, but studies at Camp Verde belie the thought. There, ARS researchers grew marketable trees averaging 5 to 7 feet in height in 3 years using runoff farming. The area averages only 12 inches of rainfall per year.

In runoff farming, water harvesting is

used to concentrate the local, sparse precipitation until adequate moisture is obtained in the soil to grow a crop. Thus, a portion of the land is sacrificed—there's a lot of extra land in semiarid areas—to obtain enough water for growing a crop on the remainder.

To improve runoff, soil in the contributing areas is usually treated with a substance to prevent infiltration. At Camp Verde, the researchers used common table salt on one area and a residual grade wax—a cheap byproduct of the petrochemical industry—on the other.

Soil scientist Dwayne H. Fink and plant physiologist William L. Ehrler (now retired) set out to determine if any marketable crop could be produced in such an area. They chose two conifers—Quetta pine and Arizona cypress—as crops most likely to succeed.

After only 3 years, nearly 90 percent of Arizona cypress "watered" by wax-treated acreage reached salable size (see table). The slower growing Quetta pines receiving water from salt-treated acreage achieved the same result after 4 years. Half of the pines in the wax treatment died, and more than two-thirds of the cypress in the salt treatment were unsuitable for sale.

Disposition of Christmas Trees at Camp Verde, Ariz.

	Cumulative salable (%)				
	after— `			Died	Culled
Treatment	3 yr	4 yr	5 yr	(%)	(%)*
Wax					
Ariz. cypress	89	89 24	89	3	8
Quetta pine	11	24	51	47	2
Salt					
Ariz. cypress	24	24	29	3	68
Quetta pine	24	88	92	4	4

^{*} Culls not suitable for sale

"Results of our Christmas tree studies indicate that a semidesert area can grow an economically valuable crop using only water supplied by runoff-farming practices. This technique will become more important as water from conventional irrigation sources either dries up or becomes prohibitively expensive, or as marginal land in arid areas is pressed into crop production," says Fink. He hopes to evaluate runoff farming for jojoba next.

Dwayne H. Fink is located at the U.S. Water Conservation Laboratory, 4331 E. Broadway Rd., Phoenix, Ariz. 85040.

—Paul Dean, Oakland, Calif.
■

Gypsy Moths—''Next Time, We'll Be Ready''

People in the Northeast are asking, "Where did all the gypsy moths go?" After several devastating spring assaults on forest and backyard trees and shrubs, populations of gypsy moths have crashed. "But, they will be back," says ARS entomologist Ralph Webb. "History tells us they will resurge in a few years in greater numbers."

Webb and other scientists with ARS, state experiment stations, and the Forest Service want to be ready for the moth's return. They are working on methods for making the insects sick, sterile, sexcraved, or sought after by parasites. Affected states will combine some or all of these methods to augment chemical spraying in an integrated attack on the creature.

When populations crash, some gypsy moth caterpillars starve to death—they eat themselves into the "empty pantry" of a denuded forest. Many more, however, fall victim to a buildup of disease pathogens and parasites, says Webb. Vigorous caterpillars that stripped nearly 13 million acres in 1981 have now become a far fewer lot, infested and infected with their own pests—a virus and several parasitic wasps and flies.

The parasites, introduced by ARS scientists, may still be thriving in many forest areas on the strength of the 1981 peak populations. Learning the parasites' habits helps states routinely release them into moth-infested areas or limit spraying

where parasites are helping to restrain the pest, Webb explains.

The virus has been developed into an insecticide, but commercial success hinges on transforming the slow-acting virus into a quick-kill agent, says ARS entomologist Ed Dougherty. He and coworkers are trying to alter the genetic makeup of the virus by genetic engineering or by selective propagation in a test tube. Other scientists, through immunoassay techniques, may soon develop a virus-detecting test. Extension agents or forest rangers could use the test to measure killing power of the virus in the forest.

ARS scientists have also found improved uses for the female moth's sex pheromone and sterilized male moths. Isolated, purified, and synthesized in an ARS lab several years ago, the sex pheromone can be an effective tool for upsetting gypsy moth mating. Scientists are looking for the best ways to use the lure in integrated pest management strategies.

Sterile males also upset sexual reproduction. They are useful in stemming outbreaks in new regions. Thousands of sterile males are set loose into infested forests and mate with females who then bear no young. Thanks to an ARS team working at laboratories of the Animal and Plant Health Inspection Service at Cape Cod, Mass., costs are much lower to mass-rear sterilized males for mass release.

For the backyard gypsy moth battle, aid is also on the way. When heavy forces of gypsy moths return, says Webb, the struggle may tilt more to the homeowners' favor. Webb runs experiments to evaluate gypsy moth control products currently on the market. Insecticide-impregnated tree bands and devices to destroy egg masses are being improved, he says.

Ralph Webb is located in Bldg. 470 and Ed Dougherty is in Rm. 231, Bldg. 011A, Beltsville Agricultural Research Center, Beltsville, Md., 20705.—Stephen Berberich, Beltsville, Md. ■



Mel Coplin, a quality inspector of the NASA Aircraft Program and amateur beekeeper, removes comb from the shuttle module. Photo by Richard Carson, Houston, Tex.

Space Bees []/

On Earth they are highly social, make symmetrical combs, pollinate billions of dollars worth of crops, and, of course, make honey. But how do honey bees behave in space?

A colony of Texas-reared honey bees took off aboard the April 6 shuttle and returned a week later as part of the Shuttle Student Involvement Program. A high-school student from Waverly, Tenn., now attending Tennessee Technological University, conceived the experiment, which was sponsored by Honeywell Corporation. Astronauts made video recordings of the colony for 5 minutes each day.

Although the bees were somewhat disoriented during their first day in space, they settled down to make a normal amount of comb, care for their queen, and keep things tidy around the artificial hive. The queen also laid several eggs during the mission, says Hachiro Shimanuki, chief of ARS' Bioenvironmental Bee Laboratory, Beltsville, Md., who helped design the experiment. Shimanuki and colleagues reviewed the videotape and studied the comb for abnormalities.

The bees, in fact, produced two types of comb—a freestanding comb from their own wax and a smaller comb on a sheet of supplied wax, says John Vandenberg, an entomologist with the Beltsville lab. He and engineer David Massie compared the geometry of the zero-gravity combs with combs made by a sister colony in Houston. Honey bee comb is considered to be a marvel of structural engineering.

Zero-gravity appears to have affected the bee's precision engineering, says Vandenberg. The angle of the cells to the foundation layer varied within the comb, and the six-sided cells were not always adjacent to six other cells. There was also more variability in thickness of cell walls.

Otherwise, he says, the bees functioned normally. They were seen flying in their cramped quarters and working side-by-side on a section of the comb while a kind of bucket brigade of bees conveyed food to them. The undertaker crew deposited dead bees in one corner, and the ventilation crew fanned air from the screened opening into the comb.

Hachiro Shimanuki and John Vandenberg are located at Bldg. 476, Beltsville Agricultural Research Center— West, Beltsville, Md. 20705.—Stephen Berberich, Beltsville, Md. ■

The Resilient Roach Succumbs to Birth Control

Spray-on birth control is the latest chemical weapon to battle cockroaches.

The new chemical, called hydroprene, prevents the birth of offspring by mimicking the hormone that governs when young roaches become adults, says ARS entomologist Richard S. Patterson,

Gainesville, Fla. He and University of Florida entomologist Philip G. Koehler evaluated hydroprene, which was developed by Zoecon Industries, Inc., of Dallas.

"Hydroprene looks promising for controlling roaches in places where conventional spray programs have not worked well," Patterson says, "particularly large apartments, warehouses, and military complexes." During the testing, a single spraying throughout a 100-unit apartment complex cut the roach population by 95 percent after 8 months. When young roaches are sprayed, they continue to grow and live out their normal lifespans, but their matings produce no offspring, he explains.

The U.S. Environmental Protection Agency recently registered three Zoecon products containing hydroprene as the active ingredient, but the company has not yet marketed them, a spokesman says.

"Even under the best conditions, conventional roach control is not a one-shot proposition," says Patterson. Some roaches get sublethal doses; others manage to avoid sprayed surfaces; and many develop resistance to the chemicals.

"A one-two punch to overcome such problems can be built into existing roach spray products by mixing them with hydroprene," he says. The conventional spray brings the population down to an acceptable level, then hydroprene works over the next 6 or 7 months to curb the birthrate.

Besides thwarting reproduction, hydroprene produces homosexuality in adult male roaches and twisted wings in both sexes—a phenomenon that visually "marks" roaches succumbing to the chemical.

Richard S. Patterson is located at the Housefly and Stablefly Biology Research Laboratory, 1700 S.W. 23 Dr., P.O. Box 14565, Gainesville, Fla. 32604.

-Russell Kaniuka, Beltsville, Md.



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Patents

PATENTS is a regular feature of *Agricultural Research* magazine. Its purpose is to make the more than 1,200 patented inventions of the U.S. Department of Agriculture better known to businesses and individuals that might benefit from using them

If you would like further technical information on a particular invention, contact the scientist whose name and address appears at the end of each item.

If you are interested in applying to obtain the license on a patent, write to the following address for an application form and information on license provisions and licensee responsibilities: Patents Office, USDA-ARS, Office of the Administrator, Rm. 323, Bldg. 003, Beltsville Agricultural Research Center—West, Beltsville, Md. 20705.

A Method for Making Multiherbicide Granules

A simple method for encapsulating several layers of different herbicides—either living pathogens or chemicals—into one granule should be of interest to marketers of preemergence herbicides and other agricultural chemicals.

Use of the tung-oil encapsulated herbicide granules would enable farmers to control two or more classes of weeds with only one trip across the field. The process also makes it possible to combine plant pathogens, inorganic chemicals, and organic chemicals into one treatment.

The precise placement possible with

such granules reduces waste and movement of chemicals to places they're not wanted. And the large amounts of water used for spray applications aren't needed.

For further technical information contact Jim Dale, Delta States Research Center, P.O. Box 225, Stoneville, Miss. 38776. Patent Application Serial No. 555,749, "Multiphase Agricultural Herbicide Granule Method."

Low Formaldehyde Release Fabrics

A process now exists that makes it possible to use carbamate finishing agents for producing durable-press textiles (such as cotton and rayon), while reducing the amount of formaldehyde released from them. Use of the process would benefit people who work around or use methylolated-carbamate-finished fabrics. Furthermore, the fabrics produced stay wrinkle-free longer than those finished with traditionally used agents.

The invention should be of interest to chemical firms, producers of carbamates, and textiles firms.

For further technical information contact Bethlehem K. Andrews, Southern Regional Research Center, P.O. Box 19687, New Orleans, La. 70179. Patent Application Serial No. 586,618, "Process to Produce Durable Press Low Formaldehyde Release Cellulosic Textiles."

Ready Diagnosis of Bluetongue Virus

A new diagnostic reagent for identifying bluetongue virus (BTV) in sheep and cattle eliminates cross-reactions with another closely related virus—a problem that clouded diagnostic accuracy in the past.

This major improvement in bluetongue diagnosis should be of interest to chemical and medical firms specializing in veterinary products and instruments.

The process involves hybrid cells that produce monoclonal antibodies to BTV in laboratory cultures, thereby eliminating the need for experimental animals to produce the reagent.

For further technical information contact Michael M. Jochim, Arthropod-Borne Animal Disease Laboratory, P.O. Box 25327, Denver Federal Center, Denver, Colo. 80225. Patent Application Serial No. 570,155, "Monoclonal Antibodies to Bluetongue Virus Antigen."

Speeding Up Graft Unions

Growers of nursery stock and horticultural hobbyists should be interested in an invention that speeds up the growth of callus tissue at the graft union of stock and scion (see *Agricultural Research*, July 1981, p. 12).

The apparatus, constructed of 2-inch and ½-inch plastic pipe, heating cables, and foam rubber, is easy to construct and transport, and makes the use of expensive greenhouse space unnecessary. Trees are grafted outdoors during the dormant season. Since only the graft union area is heated, the scion buds remain in a dormant state.

The invention is particularly useful in grafting fruit and nut trees, shade trees and evergreens.

For further technical information contact Harry B. Lagerstedt, Clonal Germplasm Repository, 33447 S.E. Peoria Rd., Corvallis, Oreg. 97333. Patent No. 4,383,390, "Method and Apparatus for Hot-Callusing Graft Unions."